bacter spp., classic pathogens of invasive dysenteric disease, are isolated in 1–15% of cases. Vibrio species are most common in Asia. Less common bacteria are Aeromonas hydrophila and Plesiomonas shigelloides. Parasitic causes of TD include E. histolytica, G. lamblia and Cryptosporidium. Viruses such as Rotavirus and Norwalk-like Virus have been isolated from up to 12% of visitors to Latin America, Asia and Africa. The most pragmatic approach to the prevention of TD is the advice to "cook it, boil it, peel it, or forget it". Prophylaxis with antimicrobials has been shown to be of benefit in preventing TD with Trimethoprim-sulfamethoxazole and fluoroquinolones, but the routine use of these agents is generally not recommended because of sideeffects of antibiotics and the development of resistance. Antimicrobial prophylaxis can be discussed in patients with inflammatory bowel disease, immunocompromised patients (HIV-infected persons or transplant recipients) or patients for whom dehydration would be dangerous. The mainstay of treatment is adaequate rehydration with oral rehydration solution ev. supplemented with loperamide. Antimicrobials are indicated in patients with severe abdominal pain, fever or dysentery.

S06 – Efflux pumps from basic science to the patients

MoS23 Antibiotic efflux in clinically important gram-negative bacteria

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Over the past decade, antibiotic efflux systems have been discovered in a growing number of bacterial pathogens. Extensive studies on *Escherichia coli*, *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae* have led to the conclusion that efflux pumps of the RND family play an essential role in the natural resistance of these species to a vast array of inhibitors. For example, the elevated intrinsic resistance of *P. aeruginosa* to antibiotics mainly depends on the expression of two poorly specific export systems (MexAB-OprM and MexXY), the action of which is potentiated by the outer membrane permeability barrier. In pathogens exhibiting more permeable outer membranes, such as *E. coli*, efflux systems provide an efficient protection against large or hydrophobic compounds (e.g. bile acids, defensins, and macrolides).

When overexpressed following mutations in regulatory genes, RND pumps may produce clinically relevant resistance to a great many substrate antibiotics (MICs × 2–16). Such efflux mutants have frequently been reported among clinical strains of *P. aeruginosa*. According to concordant data, MexAB-OprM overproducers would account for 30 to 70% of the carbenicillin resistance in hospital strains. The virulence status of the efflux mutants however requires further investigations.

As demonstrated in *E. coli* and *P. aeruginosa*, efflux systems may have a major impact on bacterial resistance when they are combined with other mechanisms such as target alterations (DNA gyrase) or β-lactamase production. Furthermore, active export of antibiotics tends to increase the emergence of highly resistant target mutants. For all these reasons, efflux inhibitors would be invaluable drugs in the treatment of infections caused by multidrug resistant strains.

MoS24 Antibiotic efflux in clinically important Gram-positive bacteria

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Efflux pumps are now well recognised in a number of Gram-positive bacteria. The role of these pumps in normal physiology is unclear, but their increased expression produces low-level antibiotic resistance Their role, in combination with other resistance mechanisms, in producing clinical significant resistance in is becoming increasingly important. Pumps which appear to have a limited substrate range are macrolide pumps such as *mefA (Streptococcus pyogenes)*, *mefE (Streptococcus pneumoniae)* and *mreA (Streptococcus agalactiae)* or the some of tet determinants (eg, *tetK* and *tetL*). The best characterised pump in *Staphylococcus aureus* is NorA. Although Nora is associated with quinolone resistance, it can use a wide range of substrates. PmrA of *Streptococcus pneumoniae* is a pump similar to NorA. NorA and PmrA show structural and functional similarities to the efflux pumps Bmr and Blt of *Bacillus subtilis*. However, it is likely that the regulation of Nora and PmrA is different. Genome sequencing projects have indicated that homologues to existing characterised pumps are found in a wide range of bacteria. Several pumps may exist in the same strain; homologues of Bmr, Blt and EmrAB have been found in addition to Nora in a strain of *Staphylococcus aureus*. Drugs which are not affected by efflux-mediated resistance are being developed. Pump inhibitors are also being researched. Efflux pumps can contribute to intrinsic antibiotic resistance. Their inactivation in different species is being developed as a sensitive tool for screening for naturally occurring antimicrobial agents that would otherwise be effluxed from the cell.

MoS25 Efflux pumps in fungi, is it a real problem?

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Mechanisms of resistance in yeasts and fungi are various and yet incompletely worked out. Efflux pumps are implicated so far in acquired azole resistance among yeasts, particularly *Candida albicans*, as well as some other *Candida* species: *C. glabrata, C. krusei, C. dubliniensis, C. tropicalis.*

Evidence for their direct implication as mechanisms of acquired resistance in isogenic pairs or series of clinical strains with increasing levels of resistance to fluconazole (F) are the following: i) increase efflux of azoles, as well as other substrates of MDR transporters (rhodamine); ii) increase of mRNA levels for *MDR* genes (*CDR* or *MDR*); iii) *MDR* gene over-expression in *S. cerevisiae* confers resistance; iv) *MDR* gene deletion in *C. albicans* results in an hypersusceptible strain.

So far, several efflux transporter genes have been characterised, belonging either to the ATB Binding Cassette (*CDR* genes) or to the Major Facilitator (*BENr, FLU* genes) transporters, conferring various degrees of cross-resistance to azoles or other substrates.

Clinical significance of efflux pumps mediated resistance in yeasts (particularly *C. albicans*) has been largely documented mainly in Aids patients suffering from oropharyngeal candidiasis (OPC), particularly before HAART. In other clinical settings, few cases have undoubtedly been described where clinical resistance was correlated to acquired efflux mediated F resistance after a short course of F. In a rat model of endocarditis due to *C. albicans*, inhibition of the efflux resistance mechanism by cyclosporin not only restored the efficacy of F to cure infection due to a F-resistant strain, but also afforded significant better results than F alone when the strain was F-susceptible.

Thus combining clinically administrable efflux pumps inhibitors to azoles should be sought in order to improve the efficacy of this class of antifungals.

S07 – Glycopeptide resistance in *Staphylococcus aureus*

MoS26 Glycopeptide resistance in *Staphylococcus aureus* (GISA): From *in vitro* to *in vivo*

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The recent description of intermediate (homogeneous and heterogeneous) glycopeptide-resistance of *Staphylococcus aureus* (GISA) has raised concern about the importance of this potential public heath problem. However, the emergence of GISA is not a new phenomenon. Teicoplanin-resistant mutants of *S. aureus* selected *in vitro* have been reported in several laboratories since 1990. These studies have shown that teicoplanin was a more efficient selective agent than vancomycin, even if more recently a highly vancomycin-resistant mutant (MIC = 100 μg/ml) of *S. aureus* has been selected by Sieradzki *et al.* (J. Bacteriol. 1997; 179: 2557–2566) by a step-wise pressure procedure with vancomycin. The first *in vivo* emergence of teicoplanin resistance, during therapy, of *S. aureus* has also been reported in 1990 (Kaatz *et al.* J. Infect. Dis. 1990; 162: 103–108) and in several studies since. The isolation of GISA strains, often in patients which have received prolonged therapy with glycopeptides, and the possibility to select mutants from hetero-GISA with further increased resistance to glycopeptides, both *in vitro* and *in vivo*, underline the necessity to monitor carefully the serum levels of glycopeptide during therapy.

MoS27 Glycopeptide resistance in *Staphylococcus aureus*: Mechanism(s) of resistance

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Methicillin-resistant *Staphylococcus aureus* (MRSA) has become one of the most important nosocomial pathogens worldwide and poses serious infection control problems. As a rule, MRSA are multiresistant against many antibiotics and increasingly vancomycin-type glycopeptides remain the only rational treatment available. Recently, *S. aureus* strains with drastically diminished vancomycin susceptibility have been isolated, first in Japan and meanwhile in various regions of the world. Glycopeptide antibiotics exert their action by interfering with the final steps of bacterial cell wall synthesis occurring at the outside of the bacterial cell. Remarkably, their mode of action does not involve direct interference with the corresponding enzymes (transpeptidases and transglycosylases) but binding to the enzyme substrates, muropeptide building blocks. Analysis of the cell wall architecture of laboratory mutants and, more important, clinical isolates reveal that present GISA isolates can achieve reduced glycopeptide susceptibility by several unrelated changes in amount and composition of cell wall building blocks. In spite of these distinct mechanisms, a unifying scheme quite distinct from that described earlier for vancomycin-resistant enterococci will be discussed.

Detailed understanding of the molecular resistance mechanisms and the various factors involved in resistance phenotype expression is a prerequisite to influence further spread of multiresistant staphylococci and to arrive at new therapeutic alternatives.

MoS28 Glycopeptide resistance in *Staphylococcus aureus* (GISA): Measures needed for control

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The recent description in Japan, USA, France and Spain of methicillin resistant *S. aureus* (MRSA) isolates with intermediate resistance to glycopeptides is of great concern. Recent surveys from different countries show that there have been no major changes in susceptibility to glycopeptides in *S. aureus* in the last decade, but GISA strains may have been missed because these studies did not use optimal methods for their detection. In our hospital 927 patients (0.4 cases/100 adm.) acquired MRSA from Jan 1990 to Dec 1998 and the majority of infections were caused by a multiresistant epidemic Iberian clone. Screening of GISA strains has been routinely performed in all *S. aureus* (221 MRSA and 1430 MSSA) isolated since Jan 1998 to Dec 1999 and in a selected sample of 244 MRSA strains isolated in the 1990–97 period, by using plates with 2, 4 and 6 mg/L of vancomycin (V). No GISA were detected among MSSA strains. The prevalence of GISA among MRSA strains was 27.7% in 1990–94, 14% in 1995–97, 6.2% in 1998 and 1.8% in 1999. All GISA strains belonged to Iberian clone, showed MICs of 4 mg/L of V and were confirmed by population analysis profiles. The MRSA infection control program was initiated in Feb-90 and was based on: 1) Clinical laboratory-based surveillance 2) Isolation measures and reinforcement of hand washing and 3) Mupirocine treatment of MRSA carriers (pts and hcw). The monthly incidence rate decreased from a mean of 0.57 cases/100 adm. in the 1990–94 period to 0.33 cases/100 adm. in the 1996–98 period ($p = 0.004$). A decrease in V use was observed from 1995 to 1998. The decrease in the frequency of GISA strains in our hospital could be related to the reduction of glycopeptide use, the efficacy of control measures and the emergence of a new MRSA clone with MIC of 1 mg/L of V and susceptible to gentamicin.

In conclusion, specific MRSA infection control programmes, rational antibiotic policies including reduction of glycopeptide use and good practice are the key measures in reducing hospital acquired MRSA and GISA infections. Active surveillance for MRSA and GISA must be maintained.

MoS29 Therapeutic options for GISA

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Isolates of GISA reported to date have been obtained from patients who failed treatment with vancomycin. Although the number of patients is small, the repeated observation of treatment failure indicates that agents other than glycopeptides should be used to treat infections due to GISA.

The widespread concept of vancomycin as the "drug of last resort" for MRSA, has lead many to suggest that emergence of vancomycin resistance in MRSA would equate with untreatable infections. However, some GISA isolates have remained susceptible to a range of agents such as co-trimoxazole, gentamicin, tetracycline, chloramphenicol, rifampicin or quinupristin/dalfopristin. Some patients with GISA infections have apparently been successfully treated with non-glycopeptide agents, although additional treatment components such as surgical intervention make assessment of their efficacy difficult. Nevertheless, it would seem prudent to recommend that therapy of infections with GISA be guided by the results of laboratory susceptibility testing. If necessary, this should include developmental agents such as oxazolidinones. Laboratory studies with one clinical GISA isolate have suggested a synergistic interaction between beta-lactams and vancomycin The role of combination therapy with these or other agents thus merits further evaluation in vitro and in animal models. Should these or other treatment options appear favourable, their successful clinical application will depend on the rapid and reliable detection of low-level glycopeptide resistance in clinical isolates.,

S08 – Nosocomial viral infections in pediatrics

MoS30 Molecular tools for epidemiology

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The advent of molecular genotyping has provided a new basis for microbial epidemiology. The most powerful tools in virology in this context to date have been PCR based methods such as random amplified polymorphism detection (RAPD) and PCR combined with restriction enzyme analysis (PCR-REA) or DNA sequencing. More recently, multilocus sequence typing (MLST) has been introduced to provide improved resolution of bacterial isolates and contributed to databases for the identification and tracking of the global spread of virulent or drug-resistant pathogens. This approach would be most suitable for viral pathogens. Molecular methods have successfully been applied to solve questions on the epidemiology and dissemination of viral infections of miscellaneous origin. Multiplex assays detecting multiple agents and markers provide a rational basis for strain typing. The use of these techniques to monitor drug resistance, mechanisms and emergence of infectious disease by sequence polymorphisms has become increasingly important. Patterns of drug resistance markers should be correlated with the epidemiologic findings.

MoS33 Hepatitis viruses

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Hepatitis A, a childhood infection in the developing world but disappearing in Europe, readily spreads by the feco-oral route in closed settings and may remain unrecognized if anicteric. Occasional transmission has been reported via blood or blood products. However, gammaglobulin or vaccination can stop outbreaks.

Hepatitis B and C share the intravenous route of transmission and both have previously been transmitted to multi-transfused children with leukemia and thalassemia. Recently, we described two outbreaks of hepatitis C in a pediatric oncology service including 10 HCV genotype 3a cases (1990–3), and 10 HCV genotype 1b cases (1993–4). Genetic analysis showed that only a few strains had spread between the children. Many had high viral titres but remained anti-HCV negative for long periods. Our analysis indicated that transmission had occurred through violations of hygienic routines when permanent intravenous catheters were flushed with fluids from contaminated multidose vials. This was supported by a closely monitored transmission event involving a child who became HCV genotype 3a infected when treated at a ward for adults. In January 1995 all infected children were transferred to the Department of Infectious Diseases. In parallel, hygienic routines at the oncology service were thoroughly overhauled, and no further cases have occurred.

Our studies highlight the ease of spreading hepatitis C between patients with intravenous catheters and who are exposed to multidose vials, in particular if patients are immunosuppressed. Similar transmission mechanisms are also relevant for other blood borne agents like HBV, HIV and malaria, etc.